ACCURATUS —LAB SERVICES— THE ANTIMICROBIAL AUTHORITY

(For Laboratory Use Only)
Accuratus Lab Services Project # A19778
CWL 12/4/15

ACCURATUS LAB SERVICES

PROTOCOL

Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity

Test Organism:

Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)

PROTOCOL NUMBER

SRC90092115.CUST.3.PROP

PREPARED FOR

Microban International, Ltd.
11400 Vanstory Drive
Huntersville, NC 28078

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
201 W. Van Buren Street
Columbia City, IN 46725

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

September 21, 2015

Revised September 30, 2015

EXACT COPY
INITIALS MS DATE 5-11-16

Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity

SPONSOR: Microban International, Ltd.
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Huntersville, NC 28078

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TEST FACILITY: Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to document the residual activity of the test substance against the test systems (microorganisms) under the test parameters specified in this protocol.

TEST SUBSTANCE CHARACTERIZATION
According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is October 12, 2015. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of November 9, 2015. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

This document details the materials and procedure to evaluate the residual activity of a test substance on hard non-porous surfaces based on the US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces and EPA guidance provided February-April 2014 (Continuous Reduction Test Recommendations). This study design may be used to support public health claims. The study is conducted under EPA (40 CFR Part 160) Good Laboratory Practices (GLP) test conditions.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing. If the Sponsor requests a repeat test, they will be charged for an additional test. Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services. The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The United States Environmental Protection Agency (US EPA) requires antimicrobial claims to be supported by relevant test systems (microorganisms). The procedure described was based on US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces and EPA Guidance provided February-April 2014 (Continuous Reduction Test Recommendations) and approved by EPA for the Sponsor under an EPA New Protocol Review File Symbol 42182-PA-3 (EPA Decision 493252 dated November 5, 2014 entitled Protocol for Residual Self-Disinfecting Activity). For products which meet the OCSPP 810.2200 requirements for hospital disinfection, this study design may be used to support the addition of a residual disinfection claim for healthcare settings.

In accordance with EPA approved protocol, the required bacterial test systems for this study are *S. aureus* (ATCC 6538), *Enterobacter aerogenes* (ATCC 13048) and *Pseudomonas aeruginosa* (ATCC 15442). Additional bacteria may be selected for testing (e.g. E. coli, MRSA or VRE).

TEST PRINCIPLE

This protocol describes the microorganisms, equipment, data collection, procedures and controls. This method includes a regimen by which each treated surface undergoes specific wear exposures to demonstrate residual efficacy of the test product.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Vancomycin Resistant *Enterococcus faecalis* - VRE	51575	Fluid Thioglycollate Medium (FTM)	35-37°C, aerobic

The test organisms to be used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Preparation of Carriers

- 1" x 1" glass carriers are visually screened prior to use in the study and flawed carriers are discarded.
- Prior to the test, carriers are rinsed with 95% ethanol to remove oil and film.
- Carriers are thoroughly rinsed using multiple tap-water rinses followed by a two deionized water rinses, then allowed to air dry.
- Carriers are decontaminated by autoclave sterilization and then aseptically transferred to sterile Petri plates lined with 1 layer of Whatman No. 2 filter paper.

Preparation of Test Culture (compliant with AOAC Use-dilution Method (2013) for *S. aureus* and *P. aeruginosa*):

- An isolated colony is transferred from the most recent monthly working stock transfer to 10 mL TSB and incubated at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria. A minimum of 3 consecutive daily transfers are made by transferring a loopful of the previous transfer into 10 mL TSB, prior to inoculating the Initial Inoculation Culture, Reinoculation Culture or Final Test Culture.
- The Initial Inoculation Culture (transfer ≥4) is incubated for 48-54 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria. The culture is vortexed for 3-4 seconds and allowed to sit for 15±1 minutes. The culture is diluted in sterile deionized water and supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^6 CFU/carrier. The final FBS supplemented suspension is vortexed for 3-4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.
- The Reinoculation Culture (transfer ≥4) is incubated for 18-24 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria. The culture is vortexed for 3-4 seconds and allowed to sit for 15±1 minutes. The culture is then diluted in sterile deionized water and is supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^4 CFU/carrier. The final FBS supplemented suspension is vortexed for 3-4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.

- The Final Test Culture (transfer ≥4) is incubated for 18-24 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria. The culture is vortexed for 3-4 seconds and allowed to sit for 15±1 minutes. The culture is diluted in sterile deionized water and supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^6 CFU/carrier. The final FBS supplemented suspension is vortexed for 3-4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.
- Antimicrobial susceptibility testing will be performed utilizing a representative culture to verify the antimicrobial resistance pattern stated. The Kirby Bauer method will be used to verify the antimicrobial susceptibility pattern according to the current version of Standard Operating Procedure CGT-0048. The results of the antimicrobial susceptibility testing will be included in the final report.
- In addition, antibiotic sensitivity testing will be performed using a representative culture to verify the stated antibiotic resistance pattern. The testing will be performed by a qualified third party lab, such as the University of Minnesota Physicians Outreach Laboratory in Minneapolis, Minnesota. Testing will not be performed under EPA Good Laboratory Practices (40 CFR Part 160) and will be exempt from the GLP compliance statement.

Exposure of Abrasion and Non-Abrasion Control Carriers to Control Substance

- Abrasion and Non-Abrasion Control Carriers (a subset of all inoculated carriers) are treated with sterile 0.01% Triton X-100 solution by treating in the same manner as test carriers. These controls are to be performed for the longest exposure time only.
- The solution on the carriers after treatment is allowed to dry uncovered at approximately 20-23°C, targeting 45-48% relative humidity in a humidity controlled chamber for 30 minutes, or until visually dry.

Exposure of Test Carriers to Test Substance

- Four test carriers (per lot, per microorganism) are treated by spray application with the test substance. Set sprayer to "mist" setting where possible. Each carrier is treated according to the study sponsor's instructions.
- After treatment, the test substance on the carriers is allowed to dry at approximately 20-23°C, targeting 45-48% relative humidity, in a humidity controlled chamber with lids ajar for up to 1 hour such that the inoculation of carriers begins no longer than 1 hour after treatment of the carrier.

Carrier Inoculation with "Initial Inoculation Culture"

- 0.010mL of the "Initial Inoculation Culture" is spread to within 1/8 inch of the surface edge of each test and control carrier with a micropipette tip bent to approximately 45° angle.
- All inoculated carriers are dried uncovered at 35±2°C for 30-35 minutes, or until visibly dry.

Abrasions and Re-inoculations

- Test Carriers and Abrasion Control Carriers undergo a wear and re-inoculation regimen including a series of at least 12 wear cycles and 11 re-inoculation cycles to support a 24 hour residual disinfection claim. The Non-Abrasion Control Carriers do not undergo the wear cycling. The table on the following page summarizes the manipulations of all carriers in the study. Only one weigh boat is to be used on the wear tester. Glass spacers, 4"x4" will be used and switched out every cycle.
- Abrasions are conducted at room temperature and humidity, with measurements taken and recorded daily. Between abrasions, carriers are returned to a humidity controlled chamber uncovered at approximately 20-23°C and targeting 45-48% relative humidity.
- The weights of the fully assembled abrasion boats are recorded for GLP testing, prior to initiation of the wear and re-inoculation regimen and must equal 1084±1.0g
- The abrasion tester is set to a speed of 2.25 to 2.5 for a total surface contact time of approximately 8-10 seconds, for one complete abrasion cycle. Each abrasion cycle in this test equals four (4) passes, one pass to the left and one return pass to the right followed by another pass to the left and another return pass to the right. Orient carriers with machined side against glass spacers and ensure carriers sit flush with spacers.
- All surfaces in contact with carriers on the abrasion tester are decontaminated with ethanol and allowed to dry completely between each set of surface wears to prevent carryover contamination.
- The foam liner and cotton cloths on the abrasion tester are replaced between each set of abrasions.
- After each complete set of abrasions are conducted (all control and test carriers abraded), the carriers are allowed to sit undisturbed for at least 15 minutes.

- Control and test carriers are then re-inoculated with 0.010mL of the re-inoculation culture, spread with a bent needled one carrier at a time, within 1/8 inch of the surface edge and returned to the humidity controlled chamber uncovered at approximately 20-23°C and targeting 45-48% RH for a minimum of 30 minutes or until completely dry prior to initiation of the next set of abrasions.
- Cotton cloths, used as part of wet abrasions, are prepared individually prior to each wet abrasion cycle by spraying the cloth with sterile deionized water using a Preval sprayer, from a distance of 75±1cm for no more than 1 second and used immediately.

Table 1. Example of procedure timeline and target concentrations for a 24hr Residual Claim

Procedure Timeline (Hours)	**Abrasion/Re-Inoculation Procedure**	**Target CFU/carrier**
0	Test Substance Application and Drying	Not Applicable
1-2	Initial inoculation of Test and Control Carriers	10^6
2 - >24	Dry Abrasion (Wear #1)	10^4 with each reinoculation
	Reinoculation(1)*	
	Wet Abrasion (Wear #2)	
	Reinoculation(2)*	
	Dry Abrasion (Wear #3)	
	Reinoculation(3)*	
	Wet Abrasion (Wear #4)	
	Reinoculation(4)*	
	Dry Abrasion (Wear #5)	
	Reinoculation(5)*	
	Wet Abrasion (Wear #6)	
	Reinoculation(6)*	
	Dry Abrasion (Wear #7)	
	Reinoculation(7)*	
	Wet Abrasion (Wear #8)	
	Reinoculation(8)*	
	Dry Abrasion (Wear #9)	
	Reinoculation(9)*	
	Wet Abrasion (Wear #10)	
	Reinoculation(10)*	
	Dry Abrasion (Wear #11)	
	Reinoculation(11)*	
	Wet Abrasion (Wear #12)	
≥24 - 48	Determination of Residual Activity (Reinoculation 12)	10^6

Test and Control Carrier Wear and Re-inoculation Regimen

Determination of Residual Activity

- Residual activity is determined for all Test and Abrasion Control carriers after the last of the 12 wear and 11 re-inoculation cycles, and at least 24 hours but not more than 48 hours after the product application.
- Carriers are sequentially inoculated with 0.010mL of the "Final Test Culture" at an appropriate interval, spreading the inoculum to within 1/8 inch of the edge, and then letting stand for the Sponsor requested contact time. Start and stop times are recorded.
- After the contact time has elapsed, carriers are aseptically transferred into vessels containing 10 mL of neutralizer broth.
- Samples are sonicated for 20±2 seconds in a sonicating waterbath. The samples are then sufficiently vortexed.
- The Abrasion and Non-Abrasion Control samples are serially diluted ten-fold in sterile diluent. Duplicate 1.0 mL aliquots of 10^{-2} through 10^{-5} will be spread plated within approximately 30 minutes of their transfer to the neutralizer broth.
- The test carriers are serially diluted ten-fold in sterile diluent. Duplicate 1.0 mL aliquots of 10^{0} through 10^{-3} will be spread plated within approximately 30 minutes of their transfer to the neutralizer broth.
- Plates are incubated for 48-54 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria.

Inoculum Concentration Determinations

- The concentrations (CFU/mL) of the Initial Inoculation Culture, Reinoculation Culture(s), and the Final Test Culture are determined by serial dilution in diluent and plating in duplicate.
- Plates of the test microorganisms are incubated for 48-54 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria.

EXPERIMENTAL CONTROLS

Neutralization Validation Control

- For each organism tested, duplicate test surfaces are treated with the test product according to study Sponsor directions along with duplicate test surfaces treated with control solution (0.01% Triton-X).
- Neutralization Validation test surfaces are treated and dried on Day 1 (i.e. in parallel with test and control surfaces that will undergo wear and re-inoculation regimen) and allowed to sit undisturbed for the duration of the study.
- Treated and control test surfaces are aseptically transferred to 10 mL neutralization broth during the "Determination of Residual Activity" portion of the study.
- Neutralized carriers are inoculated with 0.100 mL of a dilute suspension of Final Test Culture, obtained via serial dilution in diluent, to yield ≤300 CFU/mL.
- A separate 10 mL neutralization broth vessel is inoculated with 0.100 mL of the same dilute suspension and serves as the inoculum control.
- Neutralized samples are sufficiently vortexed and held for 5±1 minutes.
- After the specified hold time, duplicate 1 mL aliquots are removed from each vessel and spread plated to determine viable CFU/mL.
- The effectiveness of the chosen neutralizer is validated if the counts recovered from the treated carriers are within 0.5 $\log_{10}$ of the control carriers.

Initial Inoculation Carrier Controls

- Two sterile carriers are inoculated with the Initial Inoculation Culture and recovered immediately.
- Carriers are harvested and enumerated following the steps detailed in the "Determination of Residual Activity" section of the protocol using appropriate dilutions for plating.

Inoculated Carrier/Initial Inoculum Viability Control

- An additional 2 carriers per microorganism are inoculated with the initial inoculation culture and dried along with the test and control carriers for each test microorganism. After the dry time the carriers are harvested in 10 mL neutralization broth and vortexed for 10 seconds ± 2 seconds. The vessels are incubated with the plates for 48-54 hours at 30±2°C for *E. aerogenes* and 35±2°C for other bacteria.

Reinoculation Carrier Control

- Two sterile carriers are inoculated upon initial use of each prepared Re-inoculation Culture and recovered immediately.
- Carriers are harvested prior to initiating abrasions and enumerated following the steps detailed in "Determination of Residual Activity" section using appropriate dilutions for plating.

Purity Control

- An isolation streak is performed for each test culture to verify culture purity.

"Soil" Sterility Control

- 0.100 mL of "soil" is plated to appropriate agar for sterility confirmation and incubated alongside the test to verify sterility.

Media/Diluent Sterility Control

- A plate or aliquot of all media (growth and enumeration media) and diluents is incubated alongside the test to verify media sterility.

Carrier Sterility Control

- One sterile, uninoculated, untreated carrier is harvested in 10 mL neutralization broth. The vessel is incubated alongside the test. Presence of growth is determined by change of color or turbidity of the neutralization broth.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
To be defined as a residual disinfectant for healthcare use, the test product must: meet the OCSPP 810.2200 requirements for a hospital disinfectant, and in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 5 $\log_{10}$ or 99.999% at a contact time of ≤10 minutes.

Success Criteria
The experimental success (controls) criteria follow:

1. In the Neutralization Control, test substance treated carrier counts must be within 0.50 $\log_{10}$ of the control treated carrier counts.
2. The media sterility controls are negative for growth.
3. The purity "isolation streaks" demonstrate a pure culture of test microorganism as evidenced by colony morphology.
4. The carrier sterility controls are negative for growth.
5. The soil sterility control is negative for growth.
6. The Initial Inoculation Carrier Control must have a minimum of 1×10^6 CFU/carrier.
7. The Re-Inoculation Carrier Control carriers must have a minimum of 1×10^4 CFU/carrier.
8. The Final Abrasion Control must have a minimum of 1×10^6 CFU/carrier.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-porous Surfaces. Protocol number 01-1A.

U.S. Environmental Protection Agency Approved Microban Protocol for Residual Self-Disinfecting Activity, EPA Decision Number 493252, accepted November 5, 2014.

DATA ANALYSIS

Calculations

$$\text{CFU/mL for initial suspension} = \frac{\text{(average CFU/plate at the dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$$

$$\text{CFU/carrier} = \frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$$

- The Geometric Mean of the number of microorganisms surviving on four control surfaces or four test surfaces = Antilog (Log_{10} X1 + Log_{10} X2 + Log_{10} X3 + Log_{10} X4)/4, where X equals the number of microorganisms surviving per carrier.

- The Percent Reduction of microorganisms surviving on test surfaces over microorganisms surviving on parallel Abrasion Control surfaces = [(Geometric Mean of Abrasion Control surfaces – Geometric Mean of test surfaces)/Geometric Mean of abrasion control surfaces] x 100

(F) Insert PO 10-16-15

STUDY INFORMATION

(All blank sections are completed by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance/Name and Batch Number - exactly as it should appear on final report):
Firebird 130, Lots 150611-001 and 150611-002
Testing at the lower certified limit (LCL) is required for registration, no aged batch is necessary.

Product Description:
☑ Quaternary ammonia ☑ Other Alcohol

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
~0.54% quat; 66.5% ethanol
(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth: ☐ _____
☑ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions
☑ Room Temperature
☐ 2-8°C
☐ Other_____

Hazards
☐ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
☐ As Follows:_____

Product Preparation
☑ No dilution required, Use as received (RTU)
☐ *Dilution(s) to be tested:
_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)
☐ Deionized Water (Filter or Autoclave Sterilized)
☐ Tap Water (Filter or Autoclave Sterilized)
☐ AOAC Synthetic Hard Water: _____PPM
☐ Other _____
**Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.*

Test Organisms: ☑ Vancomycin Resistant *Enterococcus faecalis* - VRE (ATCC 51575)

Carrier Number/organism:
4 test carriers, 4 control carriers (Abrasion and Non-Abrasion), and 2 Neutralization Controls

Carrier Surface Type: ☑ Glass ☐ Stainless Steel

Exposure Temperature: Ambient

Number of wear cycles: 12 **Number of Reinoculations** ☐ 5 ☑ 11

Number of wear cycle passes: 1 cycle (1 cycle will pass over the carrier **four times** – over and back.)

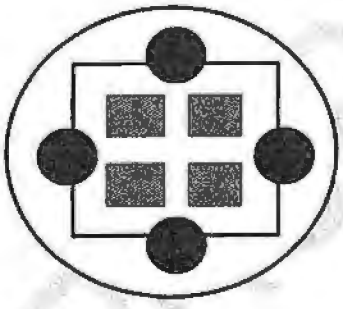
Exposure Time: 4. 5 Minutes (Time period following final carrier inoculation, prior to subculture)

Organic Soil Load:
☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
☐ No Organic Soil Load Required
☐ Other_____

TEST SUBSTANCE SHIPMENT STATUS
(This section is for informational purposes only.)
☒ Test Substance is already present at Accuratus Lab Services.
☐ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services: _____
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

PROTOCOL MODIFICATIONS
☐ Approved without modification
☑ Approved with modification
To treat carriers: To avoid filter paper buckling in the Petri dish, use the carrier treatment configuration below with 4 square carriers inside the dish with 1 piece of Whatman filter paper weighed down by black circles (may use stainless steel carriers or other appropriate sterile equipment). Prime the sprayer with at least 2 pumps to assure even flow prior to treating the carriers. Apply the test substance to all 4 replicate carriers (in the same dish) by spraying 3 pumps onto the center of the petri dish, the nozzle of the trigger spray will be approximately 6"-8" above the carrier surface at approximately a 45° angle.
Prepare the Initial and Disinfectant inoculum to target the minimum (6.00 $\log_{10}$ CFU/carrier)



PROTOCOL ATTACHMENTS
Supplemental Information Form Attached - ☐ Yes ☑ No

TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (Identity, purity, strength, solubility, as applicable) of the test lots

☑ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☑ A Certificate of Analysis (C of A) has been or will be provided for each lot of test substance to be appended to the report.
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:

_____

☐ Test has been or will be conducted by another facility under protocol or study #:

_____

☐ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

☑ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:

_____

☐ Test has been or will be conducted by another facility under protocol or study #:

_____

☐ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.

APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Rhonda Jones TITLE: Agent

SIGNATURE: R Jones DATE: 10-16-15

PHONE: (260) 244 - 6270 FAX: (260) 244 - 6273 EMAIL: rjones@srcconsultants.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Gina Sloan, SRC Staff, James Haynie

Accuratus Lab Services:

NAME: Matthew J Sathe
Study Director

SIGNATURE: Matthew Sathe DATE: 12-2-15
Study Director